

Claims

- 5 1. An expression cassette, comprising  
a polynucleotide sequence encoding a polypeptide including an HIV *Gag*  
polypeptide, wherein the polynucleotide sequence encoding said *Gag* polypeptide  
comprises a sequence having at least 90% sequence identity to the sequence presented as  
either nucleotides 844-903 of Figure 1 (SEQ ID NO:1) or nucleotides 841-900 of Figure  
2 (SEQ ID NO:2).
- 10 2. An expression cassette, comprising  
a polynucleotide sequence encoding a polypeptide including an HIV *Gag*  
polypeptide, wherein the polynucleotide sequence encoding said *Gag* polypeptide  
comprises a sequence having at least 90% sequence identity to the sequence presented as  
Figure 1 (SEQ ID NO:3) or Figure 2 (SEQ ID NO:4).
- 15 3. The expression cassette of claim 2, wherein said polynucleotide sequence  
encoding a polypeptide including an HIV *Gag* polypeptide comprises a sequence having  
at least 90% sequence identity to the sequence presented as Figure 1 (SEQ ID NO:3).
- 20 4. The expression cassette of claim 2, wherein said polynucleotide sequence  
encoding a polypeptide including an HIV *Gag* polypeptide comprises a sequence having  
at least 90% sequence identity to the sequence presented as Figure 2 (SEQ ID NO:4).
- 25 5. The expression cassette of claim 2, wherein the polynucleotide sequence  
encoding said *Gag* polypeptide consists of a sequence having the sequence presented as  
Figure 1 (SEQ ID NO:3).

6. The expression cassette of claim 2, wherein the polynucleotide sequence encoding said *Gag* polypeptide consists of a sequence having the sequence presented as Figure 2 (SEQ ID NO:4).

5           7. The expression cassette of claim 2, wherein said polynucleotide sequence further includes a polynucleotide sequence encoding an HIV *protease* polypeptide.

8. The expression cassette of claim 2, wherein said polynucleotide sequence further includes a polynucleotide sequence encoding an HIV *polymerase* polypeptide.

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9. The expression cassette of any of claim 2, wherein said polynucleotide sequence further includes a polynucleotide sequence encoding an HIV *polymerase* polypeptide, wherein the sequence encoding the HIV *polymerase* polypeptide is modified by deletions of coding regions corresponding to reverse transcriptase and integrase.

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10. The expression cassette of claim 9, wherein said polynucleotide sequence preserves T-helper cell and CTL epitopes.

11. An expression cassette, comprising a polynucleotide sequence encoding a polypeptide including an HIV *Env* polypeptide, wherein the polynucleotide sequence encoding said *Env* polypeptide comprises a sequence having at least 90% sequence identity to the sequence presented as nucleotides 1213-1353 of Figure 3 (SEQ ID NO:5).

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12. The expression cassette of claim 11, wherein the polynucleotide sequence encoding said *Env* polypeptide further comprises a sequence having at least 90% sequence identity to the sequence presented as nucleotides 82-1512 of Figure 3 (SEQ ID NO:6).

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13. The expression cassette of claim 11, wherein the polynucleotide sequence encoding said *Env* polypeptide further comprises a sequence having at least 90% sequence identity to the sequence presented as nucleotides 82-2025 of Figure 3 (SEQ ID NO:7).

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14. The expression cassette of claim 12, wherein the polynucleotide sequence encoding said *Env* polypeptide further comprises a sequence having at least 90% sequence identity to the sequence presented as nucleotides 82-2547 of Figure 3 (SEQ ID NO:8).

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15. The expression cassette of claim 11, wherein the polynucleotide sequence encoding said *Env* polypeptide further comprises a sequence having at least 90% sequence identity to the sequence presented as nucleotides 1-2547 of Figure 3 (SEQ ID NO:9).

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16. An expression cassette, comprising a polynucleotide sequence encoding a polypeptide including an HIV *Env* polypeptide, wherein the polynucleotide sequence encoding said *Env* polypeptide comprises a sequence having at least 90% sequence identity to the sequence presented as nucleotides 1513-2547 of Figure 3 (SEQ ID NO:10).

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17. An expression cassette, comprising a polynucleotide sequence encoding a polypeptide including an HIV *Env* polypeptide, wherein the polynucleotide sequence encoding said *Env* polypeptide comprises a sequence having at least 90% sequence identity to the sequence presented as nucleotides 1210-1353 of Figure 4 (SEQ ID NO:11).

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18. The expression cassette of claim 17, wherein the polynucleotide sequence encoding said *Env* polypeptide further comprises a sequence having at least 90% sequence identity to the sequence presented as nucleotides 73-1509 of Figure 4 (SEQ ID NO:12).

19. The expression cassette of claim 17, wherein the polynucleotide sequence encoding said *Env* polypeptide further comprises a sequence having at least 90% sequence identity to the sequence presented as nucleotides 73-2022 of Figure 4 (SEQ ID NO:13).

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20. The expression cassette of claim 17, wherein the polynucleotide sequence encoding said *Env* polypeptide further comprises a sequence having at least 90% sequence identity to the sequence presented as nucleotides 73-2565 of Figure 4 (SEQ ID NO:14).

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21. The expression cassette of claim 17, wherein the polynucleotide sequence encoding said *Env* polypeptide further comprises a sequence having at least 90% sequence identity to the sequence presented as nucleotides 1-2565 of Figure 4 (SEQ ID NO:15).

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22. An expression cassette, comprising a polynucleotide sequence encoding a polypeptide including an HIV *Env* polypeptide, wherein the polynucleotide sequence encoding said *Env* polypeptide comprises a sequence having at least 90% sequence identity to the sequence presented as nucleotides 1510-2565 of Figure 4 (SEQ ID NO:16).

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23. An expression cassette, comprising a polynucleotide sequence encoding a polypeptide including an HIV *Env* polypeptide, wherein the polynucleotide sequence encoding said *Env* polypeptide consists of a sequence having the sequence presented as Figure 3 (SEQ ID NO:9) or Figure 4 (SEQ ID NO:15).

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24. A recombinant expression system for use in a selected host cell, comprising, an expression cassette of claim 1, and wherein said polynucleotide sequence is operably linked to control elements compatible with expression in the selected host cell.

25. The recombinant expression system of claim 24, wherein said control elements are selected from the group consisting of a transcription promoter, a transcription enhancer element, a transcription termination signal, polyadenylation sequences, sequences for optimization of initiation of translation, and translation termination sequences.

26. The recombinant expression system of claim 24, wherein said transcription promoter is selected from the group consisting of CMV, CMV+intron A, SV40, RSV, HIV-Ltr, MMLV-ltr, and metallothionein.

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*Sub*  
*24*  
27. A cell comprising an expression cassette of claim 1, and wherein said polynucleotide sequence is operably linked to control elements compatible with expression in the selected cell.

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28. The cell of claim 27, wherein the cell is a mammalian cell.

29. The cell of claim 28, wherein the cell is selected from the group consisting of BHK, VERO, HT1080, 293, RD, COS-7, and CHO cells.

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30. The cell of claim 29, wherein said cell is a CHO cell.

31. The cell of claim 27, wherein the cell is an insect cell.

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32. The cell of claim 31, wherein the cell is either *Trichoplusia ni* (Tn5) or Sf9 insect cells.

33. The cell of claim 27, wherein the cell is a bacterial cell.

34. The cell of claim 27, wherein the cell is a yeast cell.

35. The cell of claim 27, wherein the cell is a plant cell.

36. The cell of claim 27, wherein the cell is an antigen presenting cell.

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Sub B  
37. The cell of claim ~~36~~, wherein the lymphoid cell is selected from the group consisting of macrophage, monocytes, dendritic cells, B-cells, T-cells, stem cells, and progenitor cells thereof.

38. The cell of claim 27, wherein the cell is a primary cell.

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39. The cell of claim 27, wherein the cell is an immortalized cell.

40. The cell of claim 27, wherein the cell is a tumor-derived cell.

Sub A 15  
A5

41. A composition for generating an immunological response, comprising:  
an expression cassette of claim ~~1~~.

42. The composition of claim 41, further comprising a *Gag* polypeptide.

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43. The composition of claim 41, further comprising an adjuvant.

44. A composition for generating an immunological response, comprising:  
an expression cassette of claim ~~1~~.

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45. The composition of claim 44, further comprising an *Env* polypeptide.

46. The composition of claim 44, further comprising an adjuvant

47. The composition of either claim 44 or 45 further comprising:

an expression cassette of claim 1.

48. The composition of claim 47, further comprising a *Gag* polypeptide.

49. A method of immunization of a subject, comprising,  
introducing a composition of claim 47 into said subject under conditions that are  
compatible with expression of said expression cassette in said subject.

50. The method of claim 49, wherein said expression cassette is introduced using  
a gene delivery vector.

51. The method of claim 50, wherein the gene delivery vector is a non-viral  
vector.

52. The method of claim 50, wherein said gene delivery vector is a viral vector.

53. The method of claim 52, wherein said gene delivery vector is a Sindbis-virus  
derived vector.

54. The method of claim 52, wherein said gene delivery vector is a retroviral  
vector.

55. The method of claim 52, wherein said gene delivery vector is a lentiviral  
vector.

56. The method of claim 49, wherein said composition delivered using a  
particulate carrier.

57. The method of claim 49, wherein said composition is coated on a gold or tungsten particle and said coated particle is delivered to said subject using a gene gun.

5 58. The method of claim 49, wherein said composition is encapsulated in a liposome preparation.

59. The method of any of claims 49-58, wherein said subject is a mammal.

10 60. The method of claim 59, wherein said mammal is a human.

61. A method of generating an immune response in a subject, comprising:  
providing an expression cassette of claim 1,  
expressing said polypeptide in a suitable host cell,  
isolating said polypeptide, and  
15 administering said polypeptide to the subject in an amount sufficient to elicit an immune response.

20 ~~62. A method of generating an immune response in a subject, comprising  
introducing into cells of said subject an expression cassette of claim 1, under  
conditions that permit the expression of said polynucleotide and production of said  
polypeptide, thereby eliciting an immunological response to said polypeptide.~~

25 ~~63. The method of claim 62, where the method further comprises administration  
of an HIV-derived polypeptide.~~

64. The method of claim 63, wherein administration of the polypeptide to the subject is carried out before introducing said expression cassette.



65. The method of claim 63, wherein administration of the polypeptide to the subject is carried out concurrently with introducing said expression cassette.

66. The method of claim 63, wherein administration of the polypeptide to the  
5 subject is carried out after introducing said expression cassette.

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